Mathematical modelling of metabolism

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Mathematical models of the cellular metabolism have a special interest within biotechnology. Many different kinds of commercially important products are derived from the cell factory, and metabolic engineering can be applied to improve existing production processes, as well as to make new processes available. Both stoichiometric and kinetic models have been used to investigate the metabolism, which has resulted in defining the optimal fermentation conditions, as well as in directing the genetic changes to be introduced in order to obtain a good producer strain or cell line. With the increasing availability of genomic information and powerful analytical techniques, mathematical models also serve as a tool for understanding the cellular metabolism and physiology.

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Abbreviation
MFA metabolic flux analysis

Introduction

Mathematical modelling is a very powerful tool in physics, chemistry, and engineering for interpretation and prediction of natural phenomena and experimental results [1]. A mathematical model is always a simplification of the actual phenomenon and it is therefore possible to establish different mathematical models for the same phenomenon, depending on the objectives of the model and the available measurements [2].

With the explosion in experimental data within biology, there are many attempts to develop mathematical models for description of cellular functions — either overall function or the function of individual cellular processes. Thus, in the field of functional genomics, bioinformatics plays a prominent role in assigning function to orphan genes. Within biotechnology there is especially a focus on the cellular metabolism, as this may be exploited for the production of compounds that might find application as materials, pharmaceuticals, food additives, and so on. Besides featuring very complex networks, with interconnecting pathways that consist of hundreds of reactions, the metabolism of a living cell is also subject to control and regulatory mechanisms. These regulatory mechanisms are not completely elucidated and are therefore very difficult to quantify. Thus, the establishment of fully mechanistic models to describe the cellular behaviour in terms of its metabolism is, at least to date, not possible and all models are therefore based on simplifications. In this context, mathematical models play a crucial role in hypotheses testing, that is, they can serve as a guide to choosing among different possible regulatory structures for a specific cellular process.

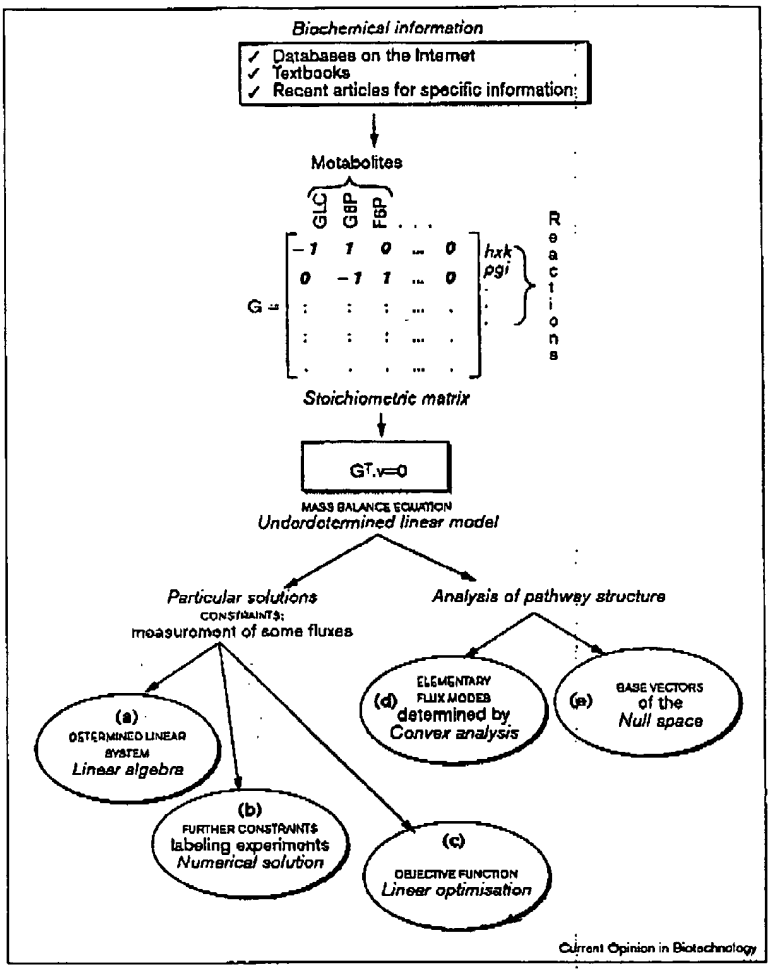
In traditional studies of fermentation processes, extracellular metabolites (such as substrates and products) have been measured, as well as the biomass concentration. The models that can be formulated based on these type of measurements are, however, highly unstructured and their application to the interpretation of cellular physiology and prediction of cellular behaviour under different cultivation conditions is quite limited. Following the advances in analytical techniques, measurement of the concentrations of intracellular metabolites and the activities of intracellular enzymes has allowed for the formulation of more structured models, that have increased the possibility for interpretation and prediction of cell physiology. Besides this, the increase in speed and power of computers has made it possible to solve the highly non-linear models that arose from the inclusion of more structure in the models. More recently, the development of new powerful analytical techniques such as DNA arrays, 2D-gel electrophoresis, and mass spectrometry has enabled a very detailed analysis of cellular function that might form the basis for a new category of mathematical models that describe the overall cellular function.

In this review, we focus on mathematical models that describe the cellular metabolism, as these models play a central role in the rapid developing field of metabolic engineering [3,4]. In our discussion, we group the models according to their structure: firstly, stoichiometric models, which are based on the time invariant characteristics of metabolic networks; and secondly, kinetic models, which are usually based on both stoichiometry and enzyme or microbial kinetics.

Stoichiometric models

Metabolic flux analysis (MFA) has been widely used for the quantification of the intracellular fluxes in the central metabolism of bacterial, yeast, filamentous fungi and animal cells (Figure 1). In MFA, mass balances over all the intracellular metabolites are used to calculate the fluxes through the different branches of the network. Hereby it is possible to get a snapshot of the metabolism under a particular condition. The fluxes can be calculated by combining measurements of a few fluxes either with linear algebra or linear optimisation [5,6]. More recently, the use of labelled substrates combined with the measurement of the labelling state of intracellular metabolites, either by NMR [7,8•] or by gas chromatography/mass spectrometry (GC-MS) [9], has been used to estimate the fluxes. When the material balances used in

Figure 1



Principles of stoichiometric modelling. Firstly, a stoichiometric matrix is defined that appropriately describes the metabolism under investigation, based on available biochemical information. Secondly, the stoichiometric matrix is multiplied by the so-called vector of reaction rates, defining the mass balance equation. Finally, algebraic manipulation of the stoichiometric matrix can be carried out by different means, depending on the objective of the analysis. (a) It is possible to impose some constraints (e.g. by measuring some fluxes) so that the system becomes determined and can be solved by simple linear algebra. In this case, the analysis is usually called flux balancing. (b) If further constraints are imposed by measuring the labelling state of some metabolites, however, it may be possible to combine flux balancing with labelling balancing and the system has to be solved numerically. In this case, a better estimation of the fluxes is attained. (c) If the system cannot be constrained to a determined one, linear optimisation can be applied to find the maximum or minimum of a suitable objective function. (d) Instead of calculating particular solutions, it is possible to gain some insight into pathway structures within a given metabolic network by applying convex analysis to determine the so-called elementary flux modes or (e) by calculating biochemically meaningful base vectors for the null space of the stoichiometric matrix. F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; GLC, glucose.

traditional MFA are combined with balances of the labelling pattern of the metabolites, the models become non-linear [10–13,14•]. The additional information supplied by measurements of the labelling pattern of the metabolites do, however, allow for a more reliable estimation of the fluxes, as well as the analysis of the pathway structures and possible reversibilities. These new features make it possible to speculate about some key points in cell metabolism, such as pathway identification and compartmentation of enzymes and metabolites, which lead to the coining of the term 'metabolic network analysis' (MNA) [14•].

Besides being applied to the investigation of cells under different environmental conditions and for studying different mutants of a particular cell (see Table 1 for some recent MFA applications), these stoichiometric models have also been applied with the aim of predicting the genotype–phenotype relationship, in order to fill in the gap that exists between DNA sequence data and functional information. Schilling *et al.* [15•] have constructed a stoichiometric model describing all known reactions in *Escherichia coli*, and have analysed the phenotypes of different deletion mutants, using linear optimisation. The predictions made by using this approach were in agreement with 60 of the 66 mutants examined. Furthermore, it was proposed that by identifying a proper set of vectors that span the null space of the stoichiometric matrix or by determining the so-called elementary flux modes by convex analysis, it is possible to assess all the capabilities of a metabolic genotype [16,17••] (Figure 1).

Stoichiometric models are clearly very powerful, but their main drawback is the limited predictive power,

Table 1

Some recent applications of stoichiometric models.

System investigated	Kind of approach	Kind of analysis	Main achievements	Reference
Serine alkaline protease production by *B. licheniformis*	MFA + linear optimisation	(a)	Design of an oxygen transfer strategy in the bioreactor for SAP production (not verified experimentally).	[42]
E. coli mutants lacking the acetate production pathway (*ackA-pta* genes)	MFA	(b)	Mutations in both *ackA-pta* and *nuo* genes (encodes for a NADH:ubiquinone dehydrogenase) are required for reduction of the flux through the pyruvate-formate-lyase reaction.	[43]
E. coli expressing the acetolactate synthase gene of *B. subtilis*	MFA	(b)	Reduction of acetate formation and increase of acetoin formation, which is less toxic to growth and heterologous protein synthesis.	[44]
E. coli expressing the *phb* operon of *R. eutropha*	MFA + linear optimisation	(b)	In order to maximise the yield of PHB, the availability both of acetyl CoA and NADPH have to be increased (experimental results were not in agreement with MFA predictions).	[45]
Pyruvate production by *T. glabrata*	MFA	(a)	Pyruvate production is mainly affected by the fluxes through pyruvate dehydrogenase and pyruvate decarboxylase reactions. A thiamine feeding strategy and the influence of dissolved oxygen on pyruvate accumulation were also investigated.	[46]
Production of IgG against human fibronectin by murine hybridoma cells	MFA	(a)	Interpretation of steady-state multiplicity (different cell concentrations at the same dilution rate) as a different utilisation of pyruvate in the TCA cycle.	[47]
Wild-type and recombinant *Z. mobilis* fermentation on glucose, fructose and xylose	MFA + labelling experiments	(a) (b)	Synthesis of ribose-5-phosphate occurs mainly via the reversible transketolase reaction. Evidence of reversible operation of ribose-5-phosphate isomerase, phosphoglucose isomerase and ribulose-5-phosphate epimerase. Identification of the xylulokinase enzyme activity as the rate controlling step for ethanol production in recombinant *Z. mobilis*.	[48]
Production of PHB in mixed cultures of *L. delbrueckii* and *A. eutrophus*	MFA	(a)	NADPH generated in the isocitrate dehydrogenase catalysed reaction in *A. eutrophus* is mainly used for α-ketoglutarate conversion into glutamate when NH_3 is abundant, whereas it is used for PHB formation when NH_3 concentration decreases.	[49]
Citrate–glucose cometabolism in *B. subtilis*	MFA	(a)	Cofeeding of citrate with glucose reduces acid formation and increases cell yield on carbon source due to an attenuation of pyruvate kinase flux.	[50]
Enhanced biological phosphorus removal (EBPR)	MFA + linear optimisation	(a)	Probably the first attempt to apply MFA to an undefined, mixed culture. Degradation of polymers and uptake or release of phosphate and acetate in an EBPR system could be predicted by the model; however, the results differed up to 55% from experimental data.	[51]

(a) Same cells under different environmental conditions. (b) Different mutants under the same environmental conditions.

which is due to the lack of regulatory information in the model formulation.

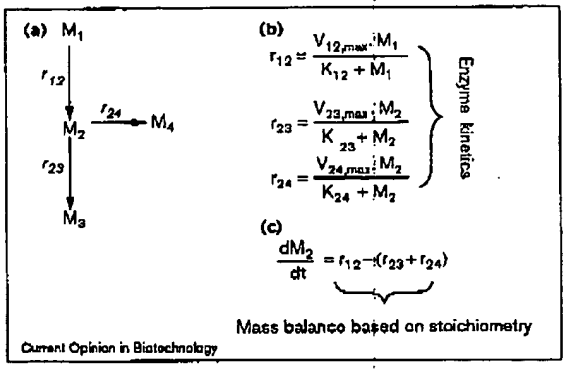
Kinetic models

When detailed information is available about the kinetics of specific cellular processes (e.g. enzyme-catalysed reactions, protein–protein interactions, or protein–DNA binding) it is possible to describe the dynamics of these processes by combining kinetics with the known stoichiometry of metabolic pathways (Figure 2). Rizzi *et al.* [18] have applied this methodology to model glycolysis in *Saccharomyces cerevisiae*. The model includes reactions of the Embden–Meyerhof–Parnas (EMP) pathway, tricarboxylic acid (TCA) cycle, glyoxylate cycle, and respiratory chain (22 material balances around metabolites and 23 rate equations for enzymes have been included) and has been successfully applied to predict the levels of intracellular and extracellular metabolites after a glucose pulse in a continuous culture of *S. cerevisiae*. The model has only been applied to a 120 s timescale, however, and phenomena such as enzyme synthesis and degradation — which would be important in larger timescales — have not been included. Besides this, regulation has only been included at the individual enzyme level and all the kinetic parameters for enzymatic activities are taken from *in vitro* experiments, which probably do not reflect the *in vivo* situation. Vaseghi *et al.* [19••] have applied the same strategy to investigate the pentose phosphate (PP) pathway in *S. cerevisiae*. The concentrations of some intracellular metabolites, such as glucose-6-phosphate and 6-phosphogluconate, as well as of the co-enzyme NADPH predicted by the model were in agreement with experimental results. By using the model, some kinetic rate equations and parameters were identified — as they could not be found directly in the literature — and it was suggested that the split ratio between the PP and the EMP pathways is related to the intracellular concentration of $MgATP^{2-}$.

Kinetic modelling has also been applied to investigation of the penicillin biosynthetic pathway in *Penicillium chrysogenum* [20–22]. The model was used for calculating the fluxes through this pathway (enzyme kinetics for 10 reactions were included), as well as the concentrations of the involved metabolites, which were in agreement with experimental results. In these studies [20–22], the models have been applied to analysis of the flux control in the pathway, which demonstrates the use of kinetic models in the field of metabolic engineering. Van Riel *et al.* [23] have established a kinetic model for the central nitrogen metabolism in *S. cerevisiae*, including variables that account for regulatory aspects. These variables represent the concentration of so-called regulators and may indicate a way — though non-mecanistic — of accounting for the control aspects of metabolism.

Another way of accounting for the regulatory aspects of cell metabolism is by applying cybernetic principles [24,25]. In this case, cybernetic variables are introduced into a kinetic model with the aim of substituting the unknown mechanistic details of the cell regulatory architecture by an objective function by supposing that the metabolism of a cell operates with a specific overall goal, such as the optimisation of growth. This approach has been used to predict the increase in flux towards threonine formation in *Corynebacterium lactofermentum*, as a consequence of genetic modifications [26]. A further way of including regulatory aspects in a mathematical model is the approach presented by Hatzimanikatis *et al.* [27], which formulates the model as a 'mixed integer linear programming' (MILP) optimisation problem. In this case, the presence or absence of different possible regulatory loops are represented by discrete variables. This approach has been applied to the investigation of genetic alterations in recombinant high-ethanol-producing *E. coli* [28•]. By using a (log)linear approximation for a non-linear model, the authors verified a qualitative agreement between the predictions of the model and experimental results. When mechanistic details are missing, neural networks [29,30] or fuzzy logic-based models [31,32] can be used with the aim of simulating metabolic behaviour; however, the amount of experimental data required to generate these models is usually very high and also less insight into the underlying mechanisms is gained. To illustrate the difficulty in mechanistically modelling particular aspects of metabolism, Wong *et al.* [33] presented a model for the lac operon — including aspects such as catabolite repression and inducer exclusion — that required several assumptions to be made. Thus, even in this very well studied system there are still some mechanistic aspects that have not been elucidated. Some other recent applications of kinetic models are listed in Table 2.

Figure 2



Principles of kinetic modelling. In this case, a simple reaction network stoichiometry (a) is used in combination with simple Michaelis–Menten type enzyme kinetics (b) to yield the mass balance over metabolite M_2, as shown (c). If the parameters V_{max} and all K_i (the Michaelis constant for reaction r_i) are known, as well as the concentration of metabolite M_1 (which may be a substrate), it is possible to calculate the time profile of metabolite M_2.

Table 2

Some recent applications of kinetic models.

System investigated	Kind of approach	Main achievements	Reference
PHB synthesis pathway in *A. eutrophus*	Investigation of the sole PHB pathway by using enzyme kinetics	It is shown that the use of oversimplified kinetic expressions, e.g. Michaelis–Menten type, is inappropriate for describing PHB synthesis, and also that parameters taken from literature should have been determined under the appropriate physiological conditions. Simulations give support to AcCoA:CoA and NADPH:NADP+ ratios exerting regulation on thiolase and reductase catalysed reactions, respectively.	[52]
Biological phosphorous removal by *Acinetobacter* sp.	Investigation of batch cultivations by structuring biomass into three compartments	The model was successfully used to predict the transient responses of *Acinetobacter* sp. cultures from aerobic to anaerobic environments, and vice versa.	[53]
Hyaluronic acid fermentation by *S. zooepidemicus*	Investigation of batch and fed-batch cultures by structuring biomass into two compartments	It was possible to predict the biomass and hyaluronic acid time profiles in aerobic and anaerobic cultures of *S. zooepidemicus*.	[54]
Production of cellulase by *T. reesei*	Investigation of batch cultivations by three types of models: unstructured, structured into three compartments, and using neural network parameter function.	The prediction capacity and the process optimisation results using the neural-network-based model were higher when compared to the unstructured and structured models tested. However, both the neural-network-based model and the unstructured model do not give any insight into the system's underlying mechanisms.	[55]

If information on all the enzymatic reactions (both equations and parameters) of the whole metabolism of an organism were available, it would in principle be possible to apply detailed modelling to interpret experimental results and predict the dynamic behaviour of cells when subjected to a determined shift in the environmental conditions and the consequences of specific genetic changes. The limitation of this kind of approach, however, is the lack of information on general regulatory aspects, as well as the fact that normally only *in vitro* parameters are available for the enzyme kinetics. *In vivo* perturbation experiments could be a way of fine-tuning these parameters, as illustrated by Rizzi *et al.* [18].

Mathematical modelling of regulatory phenomena, such as heat-shock regulation [34], synergistic eukaryotic gene activation [35•], regulation of the G1→S transition of eukaryotic cells [36], signal transduction pathways [37], and gene expression in a complex regulon [38•], can serve as a tool — which should be used together with experimental investigation — in clarifying the molecular mechanisms behind these phenomena. This may lead to combining modelling of the metabolism with modelling of signal transduction pathways.

With the aim of trying to interpret the genotype–phenotype relationship, Hatzimanikatis *et al.* [39] propose the dynamic analysis of the protein levels of an organism instead of using a set of metabolic fluxes as the output of a non-dynamic model. As it is not possible to correlate mRNA and protein levels directly [40], the sole information on gene expression generated by hybridisation on a solid surface (such as from DNA microarrays) cannot account for the functional assignment of the genome sequence of an organism. In order to illustrate the need of combining mRNA and protein levels, Hatzimanikatis and Lee [41••] applied a continuous dynamic model to describe circadian rhythmicity that agrees with experimental observations. In spite of being incipient as a result of limitations that still exist in proteome analysis, this kind of functional modelling is very promising as it really focuses on the final product of the gene, which is the protein.

Conclusions and future perspectives

Modelling of metabolism is either predicting the cellular behaviour under a different environmental condition, given a fixed genetic background, or predicting the cellular behaviour under a different genetic background, given fixed environmental conditions. In both cases, the output is the phenotype, which may be described by metabolic fluxes and/or the concentrations of transcripts, proteins or metabolic intermediates. Describing metabolism using a combination of these different characterisation forms — both at dynamic and steady-state conditions — will be important

not only for optimising biotechnological processes, but also in the field of functional genomics. The exercise of mathematically modelling metabolism and the involved regulatory phenomena *per se* can certainly serve as a tool in the elucidation of the cells' control architectures. The limitation of all approaches is still how to include regulatory and control aspects of metabolism, which are either difficult to describe or still lack biochemical characterisation. In light of this, dynamic models are not so widespread in literature, and are usually applied to the analysis of relatively small parts of the metabolism, compared to stoichiometric models, which can describe the complete metabolic network.

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